PLASMID PROFILE OF MULTI-DRUG RESISTANCE BACTERIA ISOLATED FROM AVAILABLE WATER SOURCES AND LEACHATE SAMPLES FROM DUMPSITE AT EBIRA COMMUNITIES IN EKITI NORTH SENATORIAL DISTRICT, EKITI STATE, NIGERIA

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ABSTRACT

The drinking water and leachate samples from dumpsite of rural settlement of Ekiti North Senatorial district, Ekiti State, were subjected to bacteriological and physiochemical analysis using standard microbiological techniques and Atomic Absorption Spectrophotometric method. The mean of total bacterial, coliform and enterococcal count ranged from 0.1 x 10^5 CFU/mL to 13.9 x 10^5 CFU/mL; 0.1 x 10^5 CFU/mL to 7.2 x 10^5 CFU/mL and 0.1 x 10^5 CFU/mL to 6.3 x 10^5 CFU/mL respectively for water samples; and 14.6 x 10^7 CFU/mL to 24.2 x 10^7 CFU/mL; 11.7 x 10^7 CFU/mL to 28.3 x 10^7 CFU/mL and 13.4 x 10^7 CFU/mL to 34.9 x 10^7 CFU/mL respectively for leachate samples. *Escherichia coli* had the highest percentage occurrence of (34%) while *Klebsiella* spp had the least percentage occurrence of (1%). Only 6 - 33% and 0-18% of Gram positive and Gram negative isolates were respectively resistant to ofloxacin but all the isolates were resistant to other antibiotic at varying percentages. A little above half (54%) of the total isolates exhibited multiple antibiotic resistance, some of which posses plasmids with very high molecular weight varying between 4.361Kbp and 23.130Kbp. Physicochemical and metal concentration of the water samples ranged as follow: temperature (25 - 27^{o}C), pH (6.25 - 6.70), dissolved oxygen (12.19 - 18.29mg/L), conductivity (114 - 206µhoms/cm), turbidity (0.69 - 1.01NTU), acidity (10 - 14mg/L), alkalinity (25 - 60mg/L), biochemical oxygen demand (0.62 - 11.18mg/L), hardness (6 - 80mg/L), total suspended solid (0.87 - 0.98mg/L), chloride (18 - 86mg/L), magnesium (88 - 384mg/L), calcium (7.82 - 8.91mg/L), sodium (10.96 - 11.35mg/L), potassium (15.99 - 16.18mg/L), iron (0.19 - 0.44mg/L), zinc (0.02 - 0.23mg/L), lead (0.01 - 0.02mg/L), cobalt (0.02 - 0.04mg/L) and cadmium (0.001 - 0.01mg/L). The temperature of the leachate ranged between 34 and 34.2^{o}C, pH was between 6.3 and 7.1, zinc and lead ranged between 0.02 and 0.04 while cadmium was between 0.01 and 0.02. There were no traces of copper and cobalt detected in any of the samples. This study showed that most of the water samples in Ekiti-North senatorial district are unfit for drinking and domestic use hence should be treated prior to usage.

Keywords: Drinking water, Leachate, North Senatorial, Ebira Community and Multiple Antibiotic Resistance.

INTRODUCTION

The rate at which environmental conditions serve as source of pollution to the sources of water in developing countries is a potential treat to human health, considering that, clean and

safe water is a critical component in fostering good health for all mankind (Garba *et al*., 2010; Galadima *et al*., 2011; Odeyemi *et al*., 2012a). By virtue of its importance, the United Nation has underscored 'the reduction by half, of the present world population that lack access to safe drinking water by 2015', as one of the Millennium Development Goals (MDGs) (Alao *et al*., 2011) and also, the multifaceted linkage between water quality on one hand and human health on the other is placed at the center of development policies in Nigeria (Federal Republic of Nigeria, 2004). Unfortunately, half of the world populations, especially rural settlement still do not have access to improved sanitation facilities, hence deferring them of access to quality water supplies (Gadgil and Debby, 2003). In most rural and sub-urban settlements, particularly in Nigeria, well, spring, streams or rivers and lakes thuds serve as major and direct sources of water for drinking and other domestic purposes (Oluyege *et al*., 2009), reason for which could be directly related to lack of treated (piped) water in most part of the country. Such wells and streams are prone to contamination with pathogenic microorganisms which is most dangerous pollutant, in terms of human health (Baquero *et al*., 2008). Moreover, these government-abandoned settlements are well known for sort of improper peculiarities and anthropogenic activities that contribute to the predisposition of these sources of water to contamination (Galadima *et al*., 2011); among these activities are poor waste management, poor construction of drainage, mine tailings, availability of dumpsite and latrines close to the water sources which consequently deteriorate the water through seeping and leaching (Moore *et al*., 1998; Odeyemi *et al*., 2012b). Furthermore, prevalence of animal faeces through improper livestock farming, refuses, soil run-off by rain and other dangerous substances from the dumpsite are part of the sources of pollution to the water bodies; and infection of intestinal tract like typhoid, diarrheoa, dysentery, cholera and enteric viruses are hence inevitably transmittable through the water (Aderiye *et al.*, 1992; Jiang, 2011).

Improper refuse dumping is a common attribute of rural settlements (such as villages, clans and Ebira communities) serving as major environmental problem in Ekiti State, Nigeria. This is due to lack of access of the populace to proper waste management and modern engineered landfills. This act tends to pose an expensive and inevitable cost and problems on the populace in terms of pollution of the air (Odeyemi, 2012), water resources (Odeyemi *et al*., 2012b) and general degradation of the natural environment. The refuses dumped on these sites are subjected to either groundwater underflow or infiltration from precipitation, of which, as water passes through the waste, it picks up a variety of inorganic and organic compounds, flowing out of the wastes to accumulate at the bottom of the landfill; resulting to a contaminated water termed 'leachate' which could potentially percolate through the soil (Mor *et al.*, 2006).

Leachate is a cocktail of complex effluents which contain dissolved organic matters; inorganic compounds such as ammonium, calcium, magnesium, sodium, potassium, iron, sulphates, chlorides and heavy metals such as cadmium, chromium, copper, lead, zinc, nickel (Christensen *et al*., 2001; Aderemi *et al*., 2011). This however, indicates that nearly all water sources both surface and groundwater, available for these communities are faced with potential risk of contamination; traceable to one form of populace activity or the other.
In other to assess the present condition, in terms of portability and usability, of available sources of water for people in rural settlements in Ekiti State; and ascertain the traceable possibility of their dumpsites posing detrimental effect on the water sources, present study was focused on the bacteriological and physicochemical properties of the sources of water and dumpsites available around Ebira communities in the north senatorial district of Ekiti

State. The plasmid profile of the multi-drug resistant bacteria isolated from the samples was also discussed.

METHODOLOGY

The basic area of focus in this research was the villages, clans and Ebira communities in Ekiti North Senatorial district of Ekiti State, Nigeria. Ebira community comprises mostly of immigrants from Kogi State, Nigeria; to settle down in Ekiti State, majorly for farming activities. This senatorial district comprises of five Local Government Area (LGA) which are Ido-Osi, Oye, Ikole, Ilejemeje, and Moba local government area. Apart from Ido-Osi LGA, where samples were obtained from two villages, samples were collected from three villages each in the remaining Local Government Areas. The towns and villages of focus were: Aaye (A) and Esure (B) of Ido-Osi LGA; Imojo (C), Itaji (D) and Oloje (E) of Oye LGA; Ayedun (F), Ipao (G), and Udi-Obin (H) of Ikole LGA; Iludun (I), Eda (J), and Iye (K) of Ilejemeje LGA; Ikun (L), Ikosu (M) and Igogo (N) of Moba LGA of Ekiti State.

Collection of sample

Water samples were aseptically collected from wells (W) most of which were uncovered, close to latrines, bathrooms or dumpsite; streams (S) used as/close to dumpsites; and boreholes(B) most of which were also close to dumpsites, bathrooms or toilets. Water samples were obtained in 250mL sterile bottles (with cork). Leachate samples were also obtained aseptically from the dumpsites, at different sampling points, using a sterile needle and syringe. The samples (water and leachate) collected from different sources were transported in ice to the Microbiology laboratory of Ekiti State University for analysis within 4hrs of collection.

Preparation of leachate samples

For each of the leachate sample obtained, 1ml leachate sample was diluted in 9ml of warm (≤ 45°C) sterile diluents; buffered peptone water solutions, making a ratio of 1:9. These were then incubated at room temperature for about 2-3 hours, to aid multiplication of organism present in the samples. This suspension serves as our primary inoculum.

Determination of total bacterial count

Pour plate method was used in the enumeration of total bacterial, coliform and enterococci count based on the serial dilution techniques (Olutiola *et al*., 2000). Ten-fold dilution was prepared, using 1ml of each water samples following a vigorous shaking. Aliquots of 1ml of dilution 10^{-5} and 10^{-6} of each sample were pipette into sterile labeled Petri-dish, which were then overlaid with about 20ml of molten Nutrient, MacConkey and Bile Esculin agar (45^{o}C). Determination of bacterial load of the water samples were done in triplicates. Plates were allowed to set and incubated inverted at 37^{o}C for 24 h. The plates were counted after incubation using colony counter (Gallenkamp, England) to obtain the total bacterial counts, which were calculated by multiplying the number of colony per plate by the dilution factor and recorded in colony forming unit per ml (CFU/mL) (Olutiola *et al*., 2000).Pure cultures of isolates were kept on nutrient agar slants at 12°C until used. The isolateswere identified on the basis of cellular morphology following Gram stain and results ofbiochemical testing; including catalase production, growth in 6.5% NaCl broth, haemolytic activity and motility

(Devriese *et al*., 1992). The isolates were named in reference to the Bergey's manual of determinative bacteriology (Buchanan and Gibbon, 1974).

Antimicrobial susceptibility tests

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to Cheesbrough (2006). The Gram negative bacterial isolates were tested against eight ABTEK antibiotic discs which comprised of ceftazidine (CAZ) 30µg, cefuroxime (CRX) 30µg, gentamycin (GEN) 10µg, cefixime (CXM) 5µg, ofloxacin (OFL) 5µg, augmentin (AUG) 30µg nitrofurantoin (NIT) 300µg and ciprofloxacin (CPR) 5µg. The Gram positive isolates were also tested against the following antibiotics; ceftazidine (CAZ) 30µg, cefuroxine (CRX) 30µg, gentamycin (GEN) 10µg, ofloxacin (OFL) 5µg, augmentin (AUG) 30µg, oxacillin (OXA) 10µg, cloxacillin (COX)5µg, cefotaxin (CTX)10µg. The inoculums was standardized by adjusting its density to equal the turbidity of a Barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35^{o}C for 18 h. The diameter of the zone of inhibition (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted using CLSI guideline (CLSI, 2012).

Plasmid extraction and profiling

The selection of MAR isolates was done corresponding to the number and combination of antibiotics (resistotype) they were resistant against, ranging from 4-8 resistotype. TENS protocol describe by Liu *et al*. (1995) was employed in plasmid extraction. 1.5mL of overnight culture was Spin for 1 minute in a micro-centrifuge to pellet cells. Followed by gentle decant of the supernatant leaving 50µL together with cell pellet and vortex mixed at high speed to re-suspend cells completely. 300µL of TENS was then added**.** An inverting tube was used to Mix for 3 times until the mixture becomes sticky. 150µL of 3.0M sodium acetate (pH 5.2) was then added to the preparation, followed by Vortex mixing. The preparation was spun for 5minutes in micro-centrifuge to pellet cell debris and chromosomal DNA and then the supernatant was transferred into a fresh tube; and mixed well with 900µL of ice-cold absolute ethanol. It was then spun again for 10minutes to pellet plasmid DNA. (White pellet is observed) after which the supernatant was discarded; the pellet was rinsed twice with 1ml of 70% ethanol and dry pellet. Pellet was re-suspend in 30µL of buffer or distilled water for further use. The extracted plasmid DNA was electrophoresized on 0.8% agarose gel stained with ethidium bromide and visualized by UV-transillumination according to Robins-Browne *et al*. (2004). (TENS composition: Tris 25mM, Ethyl-dimethyl tetra-amine; EDTA 10mM, Sodium hydroxide; NaOH 0.1N and Sodium dodecyl sulphate; SDS0.5 %.).

Physicochemical and mineral analysis of water

The temperature of the water and leachate samples were enumerated at the sites of collection using thermometer calibrated in degree Celsius (°C) as described by Edema *et al*. (2001) and Ademoroti (1996); electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark). Turbidity and pH were determined at site using Water Proof Scan 3+ Double Junction and HI 98311-HI 98312 (Hanna) (Wagtech International, UK). The samples were stored under deep freezing conditions or temperature of -20°C until it was analyzed. Other physicochemical characteristics determined were; hardness determined by titrimetry; total dissolved solid and total suspended solid were determined by gravimetric method; acidity, alkalinity and sulphate were determined by

titrimetry; both nitrate and phosphate were determined colorimetrically by Spectronic-20 (Gallenkamp, UK) as described by AOAC, (2005). Metal analyses were carried out using Flame Atomic Absorption Spectrophotometer (GBC Avanta version 1.31). The instrument was set to zero by running the respective reagent blanks and the calibration curves were prepared separately for each metal by running different concentration of standard solution. Average values of three replicates were taken for each determination. Manganese was also determined using atomic absorption spectrophotometer (Perkin-Elmer Model 403).

RESULTS

The bacterial density of three major sources of water (well, stream and Borehole) and leachate sample from the dumpsite in three villages in each of the five Local Government of the North senatorial district of Ekiti State were analyzed and the mean of the values obtained for each local government were computed and presented in Table 1. The mean of the total bacterial counts ranged between 0.8 x 10^5 CFU/mL and 13.9 x 10^5 CFU/mL for well water; 0.9 x 10^5 CFU/mL and 6.8 x 10^5 CFU/mL for stream water; 0.1 x 10^5 CFU/mL and 0.9 x 10^5 CFU/mL for borehole water; and 14.6 x 10^7 CFU/mL and 24.2 x 10^7 CFU/mL for leachate sample. The mean of total coliform counts obtained for all the water sources ranged between 0.1 x 10^5 CFU/mL and 7.2 x 10^5 CFU/mL and 11.7 x 10^7 CFU/mL; and 28.3 x 10^7 CFU/mL for leachate samples. While the mean of the total enterococcal counts ranged between 0.1 x 10^5 CFU/mL and 6.3 x 10^5 CFU/mL for all the water sources; and 13.4 x 10^7 CFU/mL and 34.9 x 10^7 CFU/mL for leachate sample. High bacterial density recorded for leachate samples corresponding to the water samples were also revealed in the Table (1).

A total of two hundred and twenty four (224) bacterial isolates were recovered from this research work, all of which were characterized based on their morphological and biochemical attributes and were grouped to seven (7) genera of bacteria. The percentage distribution and occurrence of representative genera in the water and leachate samples from Ebira community in North senatorial district of Ekiti State is depicted in Table 2. *Escherichia coli* had the highest occurrence of 34%, followed by *Enterococcus* spp. with 29%, *Pseudomonas* spp. with 12%, *Enterobacter* spp. with 8%, *Bacillus* spp. and *Staphylococcus aureus* had 8% each and *Klebsiella* spp. had the least frequency of 1%. Highest percentages (67%) of the bacterial isolates were recovered from the water samples with only 33% obtained from the leachate samples.

Table 1: Average microbial load (CFU/mL) of the water and leachate samples

	Samples/Microbial load											
	Well (x10^5)			Stream (x10^5)			Borehole (x10^5)			Leachate (x10^7)		
LGA	**TBC**	**TCC**	**TEC**	**TBC**	**TCC**	**TEC**	**TBC**	**TCC**	**TEC**	**TBC**	**TCC**	**TEC**
A	3.9	0.6	1.2	6.8	3.2	5.2	0.9	0.0	0.1	21.7	14.5	13.4
B	13.9	7.2	5.4	1.5	1.0	0.6	0.1	0.1	0.2	24.2	11.7	34.9
C	1.7	0.1	0.9	1.3	0.6	0.6	0.0	0.0	0.3	22.2	17.7	18.0
D	0.8	0.7	2.4	0.9	0.2	0.6	0.1	0.0	NG	21.3	28.3	17.0
E	1.9	0.5	6.3	2.2	2.9	3.9	NG	NG	NG	14.6	17.1	31.3

Keys: LGA- Local Government Area, A- Ido LGA, B- Oye LGA, C- Ikole LGA, D- Ilejemeje LGA, E- Moba LGA, NG- No growth

Table 2: Occurrence and percentage distribution of bacterial isolates from water and leachate samples

Isolates	Distribution per samples										Occurrence per samples (%)		Percentage occurrence
	A		B		C		D		E				
	W	L	W	L	W	L	W	L	W	L	Water	Leachate	
Bacillus spp.	ND	ND	5	5	ND	3	1	2	1	ND	7 (39)	11 (61)	18 (8)
Staphylococcus aureus	3	2	2	5	ND	ND	ND	ND	3	3	8 (44)	10 (56)	18 (8)
Enterococcus spp.	8	3	9	5	13	8	7	3	8	2	45 (68)	21 (32)	66 (29)
Klebsiella spp.	1	ND	2	ND	ND	ND	ND	ND	ND	ND	3 (100)	ND (0)	3 (1)
Enterobacter spp.	8	2	3	1	1	1	1	ND	ND	ND	13 (76)	4 (24)	17 (8)
Pseudomonas spp.	2	2	4	2	2	ND	3	2	9	ND	20 (77)	6 (23)	26 (12)
Escherichia coli	9	4	19	7	8	4	8	4	10	4	54 (71)	22 (29)	76 (34)
Total	31	8	44	18	24	12	20	6	31	6	150 (67)	74 (33)	224

Keys: A- Ido LGA, B- Oye LGA, C- Ikole LGA, D- Ilejemeje LGA, E- Moba LGA, W- Water, L- Leachate

The antibiotic resistance pattern of the bacteria isolated from the water and leachate samples from ebira community in North senatorial district of Ekiti State were conducted. Table 3 shows the percentage resistance pattern of Gram positive isolates, most of which demonstrated high level of resistance to ceftazidime, cefuroxime, augmentin, oxacillin and cefotaxin. Ofloxacin showed to be most effective of all the antibiotics used. The antibiotics resistance pattern of the isolates showed the following ranges; ceftazidime (83 - 92%), cefuroxime (50 - 89%), gentamycin (0 - 45%), augmentin (77 - 89%), oxacillin (33 - 64%), cloxacillin (20 - 50%), cefotaxin (17 - 56%) and the least percentages of (6 - 33%) were resistant to ofloxacin.

Table 4 shows the percentage resistance pattern of Gram negative isolates which showed the following ranges; nitrofurantoin (0 - 58%), augmentin (33 - 85%), cefixine (67 - 95%), gentamycin (33 - 42%), cefuroxime (67 - 96%), ceftazidime (33 - 88%), ciprofloxacin (0 - 35%) and the least percentages of (0 - 18%) were resistant to ofloxacin.

Table 3: Percentage antibiotic resistance of Gram positive isolates from water and leachate samples

Organisms	Number of Isolates	Percentage resistance to antibiotics (%)								Resistotype
		CAZ	CRX	GEN	OFL	AUG	OXA	COX	CTX	
Bacillus spp.	18	83	72	0	6	89	33	44	17	CAZ,CRX,AUG
Staphylococcus spp.	18	83	50	17	33	78	44	50	56	CAZ,AUG,CTX
Enterococcus spp.	66	92	89	45	9	77	64	20	35	CAZ,CRX,AUG,OXA

Keys: CAZ- Ceftazidine, CRX-Cefuroxine, GEN- Gentamycin, OFL-Ofloxacin, AUG-Augmentin, OXA- Oxacillin, COX-Cloxacillin, CTX- Cefotaxin

Table 4: Percentage antibiotic resistance of Gram negative isolates from water and Leachate samples

Organisms	Number of Isolates	Percentage resistance to antibiotics (%)								Resistotype
		NIT	AUG	OFL	CXM	GEN	CRX	CAZ	CPR	
Escherichia coli	76	34	79	9	95	39	96	79	13	AUG,CXM,CRX,CAZ
Enterobacter spp.	17	35	82	18	82	35	76	76	35	AUG,CXM,CRX,CAZ
Pseudomonas spp.	26	58	85	14	88	42	86	88	23	NIT,AUG,CXM,CRX,CAZ
Klebsiella spp.	3	0	33	0	67	33	67	33	0	CXM,CRX

Keys: NIT- Nitrofurantoin, AUG-Augmentin, OFL-Ofloxacin, CXM- Cefixine, GEN- Gentamycin, CRX-Cefuroxine,CAZ- Ceftazidine, CPR- Ciprofloxacine

A little above half (54%) of the total isolates exhibited multiple resistance with the antibiotics they were tested against. The percentage incidence of multiple antibiotic resistance among the isolates include; *Enterococcus* spp. with the highest percentage of 62%, *Staphylococcus*

(61%), *E.coli* (58%), *Pseudomonas* spp. (54%), *Bacillus* spp. (44%), *Klebsiella* spp. (33) and *Enterobacter* spp. with the least percentage of 18% (Table 5).

The result of plasmid profile and molecular weight of twenty (20) randomly selected MAR bacteria isolated from water and leachate samples showed the absence of extra-chromosomal DNA in ten (50%) of the selected MAR isolates; nine others posses one (1) plasmid with molecular weight of 24.1Kbp each. Only *Escherichia coli* 5 was detected to posses' two (2) plasmids with molecular weights of 4.4Kbp and 23.1Kbp (Table 6). The pictorial representation of the plasmid profile and molecular weight of Gram positive and Gram negative isolates are presented Figure 1(a) and 1(b) respectively.

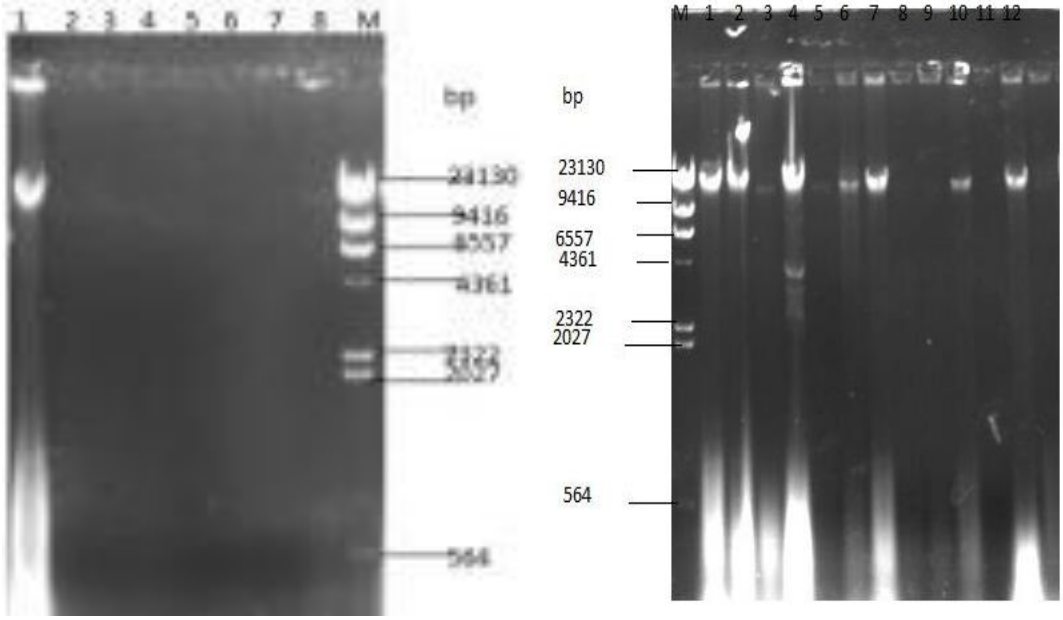
Table 5: Multiple antibiotic resistance (MAR) pattern of bacterial isolates from water and leachate samples

Organisms	Total number Isolated	Number of isolates with MAR	Percentages of MAR isolates (%)
Escherichia coli	76	44	58
*Enterobacter*spp.	17	3	18
Pseudomonas spp.	26	14	54
Klebsiella spp.	3	1	33
Bacillus spp.	18	8	44
Staphylococcus spp.	18	11	61
Enterococcus spp.	66	41	62
Total (%)	**224**	**122 (54%)**	

Table 6: Plasmid Profile and molecular weight of multiple antibiotic resistant (MAR) Bacteria from water and leachate samples

Isolates	Number of Plasmids	Molecular Weight of Plasmid (Kbp)	Antibiotics to which isolates were resistant	
			Number	Combinations
Enterococcus spp.18	-	-	4	GEN,CTR,CXC,CAZ
*Escherichia coli*64	1	23.130	4	AUG,CXM,CRX,CAZ
*Klebsiella*spp.1	-	-	4	AUG,CXM,CRX,CAZ
*Escherichia coli*18	1	23.130	4	AUG,CXM,CRX,CAZ
Enterococcus spp.38	-	-	5	CRX,CTX,CXC,AUG,CAZ
*Enterobacter*spp.13	1	23.130	5	NIT,AUG,CXM,CRX,CAZ
*Pseudomonas auriginosa*16	1	23.130	5	NIT,AUG,CXM,CRX,CAZ
Escherichia coli 2	-	-	5	AUG,CXM,GEN,CRX,CAZ
Escherichia coli 32	-	-	5	AUG,OFL,CXM,GEN,CRX
*Bacillus megatarium*2	-	-	6	CRX,CTR, CTX,CXC,AUG,CAZ
*Staphylococcus aureus*5	-	-	6	CRX,GEN,CTR, CTX,AUG,CAZ
*Enterococcus faecium*11	-	-	6	CRX,GEN,CTR, CTX,CXC,AUG
*Enterococcus faecium*22	-	-	6	CRX,GEN, CTX,OFL,AUG,CAZ
*Pseudomonas auriginosa*10	1	23.130	6	NIT,AUG,CXM,GEN,CRX,CAZ
Escherichia coli 5	2	23.130, 4.361	6	NIT,AUG,CXM,CRX,CAZ,CPR
Escherichia coli 42	1	23.130	6	NIT,AUG,OFL,CXM,CRX,CAZ
*Pseudomonas auriginosa*14	1	23.130	7	NIT,AUG,CXM,GEN,CRX,CAZ,CPR
*Staphylococcus aureus*2	1	21.130	7	CRX,GEN,CTR, CTX,CXC,AUG,CAZ
*Enterococcus faecium*42	-	-	7	CRX,GEN, CTX,CXC,OFL,AUG,CAZ
Escherichia coli 11	1	23.130	8	NIT,AUG,OFL,CXM,GEN,CRX,CAZ,CPR

Keys: CAZ- Ceftazidine, CRX-Cefuroxine, GEN- Gentamycin, OFL-Ofloxacin, AUG-Augmentin, OXA- Oxacillin, COX-Cloxacillin, CTX- Cefotaxin, NIT- Nitrofurantoin, CXM- Cefixine, CPR- Ciprofloxacine, Kbp- Kilobase pair



(a) (b)

Keys: M- Hind III DNA Molecular Weight Marker, a- Gram positive MAR isolates, b- Gram negative MAR isolates

Figure 1: Plasmid profile of MAR bacteria isolated from water and leachate samples

The average value of the physicochemical and metal concentration of the three water sources from each of the (Ebira) community in the North senatorial district of Ekiti State presented in Table 7; indicate that the water samples were colourless and odourless. The ranges of value obtained for parameters tested for were as follow; temperature (25 - 27^{o}C), pH (6.25 - 6.70), dissolved oxygen (12.19 - 18.29mg/L), conductivity (114 - 206µhoms/cm), turbidity (0.69 - 1.01NTU), acidity (10 - 14mg/L), alkalinity (25 - 60mg/L), BOD (0.62 - 11.18mg/L), hardness (6 - 80mg/L), total suspended solid (0.87 - 0.98mg/L), chloride (18 - 86mg/L), magnesium (88 - 384mg/L), calcium (7.82 - 8.91mg/L), sodium (10.96 - 11.35mg/L), potassium (15.99 - 16.18mg/L), iron (0.19 - 0.44mg/L), zinc (0.02 - 0.23mg/L), lead (0.01 - 0.02mg/L), cobalt (0.02 - 0.04mg/L) and cadmium (0.001 - 0.01mg/L). Chemicals like sulfate, nitrate, phosphate and copper were not detected in any of the water samples. The table also entails comparative values of these parameters with standard of The Federal Environmental Protection Agency (FEPA, 1991) and World Health Organization (WHO, 2006).

The temperature of the leachate ranged between 34 and 34.2^{o}C, pH was between 6.3 and 7.1, zinc and lead ranged between 0.02 and 0.04 while cadmium was between 0.01 and 0.02. There were no traces of copper and cobalt detected in any of the samples (Table 8).

Table 7: Average physiochemical and metal concentration of water samples

Parameters	Results					Standard for comparison	
	A	B	C	D	E	FEPA	WHO
Colour and odour	C/O	C/O	C/O	C/O	C/O	Nil	Nil
Temperature (^{o}C)	25.0	25.6	27.0	26.2	26.2	≤40	≤40
pH	6.70	6.25	6.44	6.43	6.50	6.0-6.9	6.5-8.5
Dissolved Oxygen (mg/L)	12.19	15.24	18.29	17.07	16.25	500	500
Conductivity (x10^{2}µhos/cm)	1.92	2..07	1.63	1.95	1.14	-	200
Turbidity (NTU)	0.69	1.01	0.73	0.75	1.00	-	5
Acidity (mg/L)	12.00	14.00	10.00	11.00	11.00	-	-
Alkalinity (mg/L)	25.00	40.00	40.00	50.00	60.00	-	-

Biochemical Oxygen Demand (mg/L)	11.18	5.08	2.03	3.66	0.62	≤10	≤10
Hardness (mg/L)	10.00	6.00	80.00	14.00	14.00	500	125
Total Suspended Solid (mg/L)	0.98	1.02	0.87	0.92	0.88	≤200	-
Sulfate (mg/L)	ND	ND	ND	ND	ND	500	250
Chloride (mg/L)	18.00	86.00	28.00	66.00	54.00	500	250
Magnesium (mg/L)	384.00	88.00	132.00	320.00	208.00	200	50
Nitrate (mg/L)	ND	ND	ND	ND	ND	50	20
Phosphate (mg/L)	ND	ND	ND	ND	ND	-	-
Calcium (Ca) (mg/L)	7.82	8.91	8.37	7.99	8.46	200	75
Sodium (Na) (mg/L)	11.01	10.96	11.35	11.06	11.02	-	-
Potassium (K) (mg/L)	16.18	15.99	16.03	16.14	16.00	-	-
Iron (Fe) (mg/L)	0.19	0.32	0.44	0.31	0.42	2.0	0.3
Zinc (Zn) (mg/L)	0.02	0.21	0.11	0.23	0.19	5.0	5.0
Copper (Cu) (mg/L)	ND	ND	ND	ND	ND	0.5	0.5
Lead (Pb) (mg/L)	0.02	0.01	0.02	0.01	0.02	<1	0.01
Cobalt (Co) (mg/L)	0.04	0.04	0.01	0.02	0.02	0.01	0.05
Cadmium (Cd) (mg/L)	ND	ND	ND	0.001	0.01	<1	0.01

Keys: A- Ido LGA B- Oye LGA C- Ikole LGA D- Ilejemeje LGA E- Moba LGA
C/O- Colourless and Odourless

Table 8: Mineral analysis of leachate samples

	Parameters/Values (mg/L)						
LGA/Samples	**Temperature**	**pH**	**Copper**	**Zinc**	**Lead**	**Cobalt**	**Cadmium**
A	34.1	7.0	ND	0.02	0.04	ND	ND
B	34.2	7.1	ND	0.02	0.02	ND	0.01
C	34.0	7.1	ND	0.04	0.02	ND	ND
D	34.0	7.0	ND	0.03	0.03	ND	0.01
E	34.2	6.3	ND	0.03	ND	ND	0.02

DISCUSSION

In recent years, a terrific amount of devotions have been directed towards water pollution in Ekiti State, Nigeria and the consequent effect on human and ecosystem. In fact, numerous articles have emphasized the alteration in the quality and portability of all major sources of water for the populace in the state; including surface water (Oluyege *et al*., 2009; Odeyemi *et al*., 2010 Odeyemi *et al*., 2012b), ground water (Odeyemi and Olanipekun, 2007; Oluyege *et al*., 2011; Odeyemi *et al*., 2011b), natural water (Odeyemi *et al*., 2013a) and even dam water (Odeyemi *et al*., 2013b) which are expected to serve as public water supply. Only few of these research works (Odeyemi *et al*., 2012b; Odeyemi *et al*., 2014) has however made attempt to trace the possible route of contamination. In this present study, the effect of a major culpability; improper dumping of refuse, peculiar to rural settlements in Ekiti State on their sources of water are showcased.

The mean of the total microbial counts of the water samples, shown in Table 1 were higher than the specified limit advocated by WHO (2006), which is an indication of high microbial contamination as a result of poor hygiene and sanitary conditions such as cloth and dish washing as well as defecating in and near the water bodies. The location of the water bodies are also of atrocious concern, considering the bushes and shrubs around the water bodies; which could possibly serve as route of contaminations (Okonko *et al*., 2008). Proximity of the water sources to dumpsite and latrines consequently deteriorate the water through seeping and leaching (Moore *et al*., 1998; Odeyemi *et al*., 2012b). Furthermore, Roohul *et al*. (2012) manifested that microbial contamination of underground water is an attribute of leakage in pipes; cross contamination with wastewater; poorly constructed well cover; short distance between water supply network and sewage supply; construction of septic tanks near wells and

drinking water supply lines; run-offs; infiltration of wastes and direct deposition of waste water through leakage. Unfortunately, most of these are parts of major peculiarities of rural settlement like the Ebira community in Ekiti State. This evident the high microbial load encountered for the well and bore-hole water analyzed in present study. Moreover, the leachate samples also showed a level of microbial density corresponding to a report of Odeyemi *et al*. (2011a) on the leachate samples from a Government managed dumpsite. This indicates that most of the unfitting dumpsites in the villages have generated hazardously enough to have gotten similar level of microbial load.

Most of the bacterial species isolated in this study were identified to belong to the members of coliform bacteria, which are Gram negative facultative anaerobes, non-spore formers that ferment lactose within 48 hours (Prescott *et al*., 2008). They have however been reported by Okonko *et al.* (2008) to be among the organisms commonly encountered in soil and aquatic environment. The high number of bacteria from the family of Enterobacteriaceae and coliform bacteria is an indication that the water samples are not potable and thus unfit for domestic use (WHO, 2006). The isolation of *Escherichia coli* from the water samples in this study is in correlation with the past studies that have presented *Escherichia coli* as a common encounter in different water sources be it rivers, streams, rain water, well water, underground water and even pipe borne water (EPA, 2002). Although, the correlation of this study with previous study is making *E.coli* to be seen as a normal flora which can be isolated from any water body as earlier reported by Zamxaka *et al.* (2004), but their pathogenecity and the consequence will forever rule the possibility out. Since the implications of this organism in water and food related pathogenic infections have been reported by different researcher (Wasteson *et al.*, 2001; Kaper *et al.*, 2004). Studies have equally proven *Enterococci* as the most relevant indicator of water quality (Teixeira and Facklam, 2003). Their presence with such occurrence in this study (Table 2), is considered an indication of faecal contamination and they are not considered 'generally recognized as safe (GRAS) (Mannu *et al*., 2003; Aparecida *et al*., 2007). All the bacteria isolated during this investigation have however been reported by Cheesbrough (2004) as potential pathogens, this fact which is significantly enough to admit that the quality of these water sources has been adversely deteriorated thereby subjecting the immune-compromised individual in the community patients to greater health risks (Yagoub and Ahmed, 2009).

It becomes more concerning to detect that the set of bacterial isolates in this study were similar to those documented to proliferate in leachate samples as reported by Odeyemi *et al*. (2011a). More so, the manner of distribution of the bacterial isolates in this study (Table 2) also observably elucidated some kind of connection between the organisms form leachate and those from water samples; seems like the latter were bore from the leachate. This however could be traceable to the proximity of these water bodies to dumpsites, through which these contaminant may have find their ways into the water via percolation, seepage or run-off; as narrated by the report of Odeyemi *et al*. (2012b). *Enterobacter* spp. and *Bacillus* spp. which are both isolated from the water and leachate samples (with almost the same distribution), is a clear picture of the connection, considering them as non-fecal coliforms mostly found in vegetation and soil as described by Schlegel (2002); this further justify the possibility of some component of topsoil seeping into the water table. *Enterococci* have also been reported to be readily recovered outdoor from water probably due to contamination by human excrement, untreated sewage and leachate seepage. Furthermore, *E.coli* which is the most occurred in this study conforms to the report of Lewis and Gattie (2002) that slated *Escherichia coli* as being able to withstand competition with other indigenous organisms with

high growth in a complex web; therefore are reported to be involved in the breaking down of organic compounds into smaller units in a complex mesh like leachate.

A more deleterious situation is to realize that the water sources meant to be serving as drinking water to the people of these settlements could also serve as reservoirs for pathogens resistant to significant number of antibiotics. Majority of isolated bacteria showed high level of resistance against quite numbers of antibiotics. The antibiotic resistance pattern encountered in this present study is in agreement with the findings of Odeyemi *et al*., (2013a) which also reported all tested isolates to be least resistance to ofloxacin. Incidence of multiple antibiotic resistant bacteria especially that they posses plasmid, in this study is in agreement with the study of Akinyemi *et al*. (2006). Going by the report of Ajayi and Akonai (2003), discharge of leachate into the water body could enhance the ingestion of resistance strain of bacteria by individuals drinking this untreated water, thereby becoming part of the human microflora, making infections caused by the organisms very difficult to treat. The uncontrolled use of antibiotics for empirical treatment of infectious diseases has been implicated as a cause of high prevalence of these antibiotics resistance (Olofsson, 2006). Another way by which these isolated bacteria can develop resistance to the various antibiotics is through the transfer of antibiotic resistant gene from one organism to another; which heightened the risk knowing that some of the isolates in this study are carry plasmid(s).

The average value of physicochemical parameters recorded for water samples from the settlement is observably considered to be within the range of permissible limit advocated by FEPA (1991) and WHO (2006). Except for magnesium and lead which are slight higher than the recommended level, all other micronutrients in the water samples were present below toxic level; making them to be of minimal threat to biological existence (IETEG, 2004). Although, even when these values are slightly higher, the enrichment of copper, calcium, lead and zinc are mostly related to the geogenic inputs through weathering/erosion and runoff from the catchment areas (Tijani *et al*., 2005). The mineral composition of the leachate samples also indicated that elements such as zinc, lead and cadmium were present at considerable limits while copper and cobalt were detected at all. This is in agreement with the findings of Ghorbani *et al*. (2002) which stressed on the toxicity of heavy metals to microorganisms. Therefore their minute amount in the leachate samples also justifies the microbial density recorded. It could be inferred from this study that, the lower the heavy metal content in the leachate samples, the higher the bacterial density, thereby validating the claim of Odeyemi *et al*. (2011a).

CONCLUSION

This present study revealed that the sources of water in the rural settlements of Ekiti North senatorial district are not safe for drinking due to contamination traceable to percolations from dumpsites around. Serious health hazards could result from consumption of this water hence, proper and adequate treatment of this water is highly required. Human attitudes such as dumping of refuse or untreated sewage and defecating in and around this water bodies should also be discouraged.

This study thereby recommends the provision of potable water, modern sanitary and sewage disposal facilities and creation of awareness to the people in the community of the risk associated with consumption of contaminated water. Also, sewage and refuse should not be dumped into the surface water bodies around the landfill site in order not to increase the nutrient availability of the water which will aid growth of organisms in the water. The present

study however challenge scientists on the need for development of new antibiotics to combat the infections caused by these resistant strains.

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